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Thermal Shock Effects on Larvae of Caddis Fly Brachycentrus americanus

JACK A. SALMELA* and RICHARD L. ANDERSON**

ABSTRACT – Wild-collected *Brachycentrus americanus* larvae were subjected to thermal shocks of 30-minute and 60-minute duration in June, September, and December of 1976. Temperatures at collection were 14.7 , 10.4 , and 1.2 C, respectively. The TL50's for both 30-minute and 60-minute shock durations ranged from 33.3 C to 34.0 C for each month, except for a 30-minute exposure in September, which had a TL 50 of 34.6 C. Larvae from a December exposure were held for 16 days to observe postexposure behavior. Feeding was reduced 50 percent among specimens exposed to temperatures 1.2 C below the 30-minute TL 50 and 3.6 C below the 60-minute TL50.

In studying influences of temperature on aquatic life, one common procedure is to collect animals from their natural habitat and to expose them to a series of high or low temperatures in the laboratory. Usually there is an acclimation period of one to three weeks in the laboratory before the exposure series is initiated. The results are usually expressed as a temperature at which 50 percent mortality (TL50) occurs within a stated time period. This method was used by Nebeker and Lemke (1968), who exposed 12 insect species to a series of high temperatures and determined 96-h TL50's. They reported a range of TL50's from 21° to 33° C for aquatic insects and correlated the values to the habitats of the test tnimals. The 96-h LC 50 for the caddis fly *Brachycentrus americanus* used in this study was 29° C (Nebeker and Lemke 1968).

A major influence on the thermal tolerance of a test animal is its thermal history. Summer-conditioned snails *(Physa virgata)* had thermal tolerance limits that were 8.4 C higher than winter-conditioned snails (McMahon 1975). The effect of naturally obtained thermal history is usually eliminated by acclimating the animals for one to three weeks in the laboratory.

An area that has received little research attention is the effect on aquatic insects of instantaneous thermal shock and short exposure. The temperature shock and short exposure periods may result in immediate death or may produce a subtle effect such as a change in behavior which may ultimately harm the animal. A recent report (Sherberger *et al.* 1977) indicated that shock treatments had to approach the upper lethal limit before lethal effects were noted. They also reported that *Isonychia* mayfly nymphs shocked at 33 C for 30 minutes did not show behavioral changes in rheotaxis, phototaxis, or substrate orientation.

This study reports both seasonal effects and thermal shock effects on larvae of the caddis fly *Brachycentrus americanus*. The specific objectives were to determine the TL50 for *B. americanus* larvae for 30-minute and 60-minute exposure periods and to determine if seasonal changes in water temperature affect the TL50.

Collection locale and methods

The larvae were collected in the Blackhoof River in June, September, and December 1976. The river is near Wrenshall, Minnesota, in the Lake Superior drainage area. Two methods of collection were used: either the substrate was stirred and the current was allowed to carry larvae into a downstream net or individual larvae were picked from the river bottom. Larvae were placed in a plastic container and transported to the laboratory. The temperature of the river was recorded.

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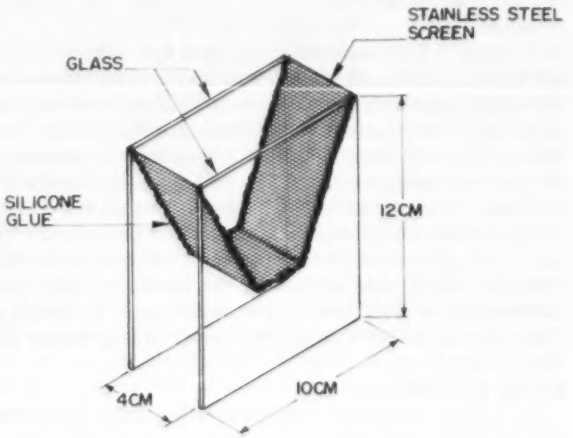


Figure 1. Stainless steel screen and glass test containers.

At the laboratory

At the laboratory larvae were placed in small glass and stainless steel screen containers (Fig. 1) immersed in three oval stainless steel tanks 90 cm long, 11.25 cm wide, and 12.5 cm deep with the water depth at 10 cm. This holding system is similar to the test apparatus described by Nebeker and Lemke (1968). The oval tanks had paddle wheels to simulate stream flow. Lake Superior water was continually added to the tanks to remove waste products and aerate the water. An overflow drain kept the water depth constant. Water temperatures in the holding tanks and in the Blackhoof River during the experimental period are presented in Table 1.

In the second test of December the oval tanks were not used, but larvae were held one day before exposure in a polyethylene pan in a controlled temperature room at 7.7°C. The pan contained 11 screen containers, one of which was a control, and five larvae were in each. After the thermal shock the larvae were held 16 days at 7.7°C to observe post-exposure feeding behavior.

Five aquaria, five temperatures

The thermal shock apparatus (Fig. 2) consisted of a series of five glass aquaria, each 16.5 cm by 20.6 cm by 30.6 cm and containing 10 liters of water. Five test temperatures (range 28.5° to 36.5° C) were obtained by passing heated water through a stainless steel tube running through each aquarium to warm the Lake Superior water. Temperatures varied by no more than ±0.5 degrees C during the test period, except for one aquarium in September and another in December which varied by ±0.7° C and ±0.9° C, respectively. The temperature in each aquarium was measured seven or eight

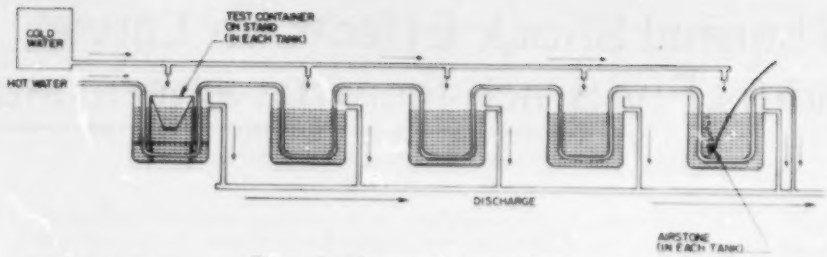


Figure 2. Diagram of the test system.

times in 60 minutes. Each aquarium was aerated to air saturation, and dissolved oxygen was determined with a modified azide analysis procedure (American Public Health Association *et al.* 1976).

Procedures

Exposures were initiated by placing two glass and screen containers as in figure 1, each containing 10 larvae, in each preheated aquarium. One container was removed from each aquarium after 30 minutes and one after 60 minutes. They were transferred back into the holding system maintained at the temperatures noted in Table 1. The control was a container of 10 larvae that remained in the holding system. About a half hour after each exposure, deaths were recorded, and the larvae were observed either 24 or 48 hours after the test. Death was defined as the absence of any body movement after several minutes of observation with prodding. The larvae were fed thawed adult brine shrimp before and after each test.

Postexposure effects determined

The effects of thermal shock on feeding were determined with the survivors of the second test in December. These insects were held in a controlled-temperature room at 7.7°C for 16 days after the exposure and were fed thawed adult brine shrimp after 4, 5, 9, and 11 days. The ratio of the number eaten to those remaining shrimp was used to describe the effect of the exposure on feeding.

Death, survival and behavior

June 1976

No significant difference in survival of larvae was observed between 30-minute and 60-minute exposures for both tests (Fig. 3). Survival decreased sharply within a small temperature range. No deaths were observed until the temperature exceeded 32.9°C. All larvae exposed at 34.5 degrees C were dead. No additional dead larvae were observed in the 48 hours after the initial count in this or later tests. Thus no delayed deaths occurred within that time period. In addition, none of the control larvae died in any test.

September 1976

The September results are similar to the June results, survival again, decreasing sharply between 33° and 34°C. No significant change in tolerance between the June and September exposures is apparent. However, the 30-minute tolerance limit increased about 1.0 degree C. (Table 2).

December 1976

As in the previous tests survival declined sharply near 33°C. Tolerance apparently did not change from the June and September tests (Table 2).

Behavioral Effects

The number of brine shrimp eaten decreased with the increase in exposure temperature (Fig. 4). The control larvae ate 100 percent of their brine shrimp. Another control, without larvae, showed that brine shrimp do not disintegrate over 16 days under the conditions of this experiment. No increase in mortality occurred during the 16-day posttreatment period.

Reduced feeding was first observed at approximately 30 degrees C. The temperature effect on survival was first seen at temperatures that were 3 degrees higher. The decrease in feeding indicates that the larvae were not accepting food. If starvation followed, the temperature tolerance of the larvae would be less than the survival data indicate.

Data in Figure 4 were processed by the Litchfield and Wilcoxon (1949) method to determine the temperatures at which 50 percent of the shrimp are eaten. Both results were compared to the averages of the tolerance limits from June, September, and December (Table 3).

Response categories described

In poikilotherms the upper lethal temperature usually is dependent on the thermal history of the test species. Three

Table 1

Temperatures (°C) of the Blackhoff River at time of collection, temperature of the holding system, and conditions of holding.

Month	Collection temperature	Pre-test holding temperature and time	Post-test holding temperature
June			
Test 1	14.7	12.1 2 days	12.2
Test 2	14.7	14.2 3 days	14.2
September	10.4	tested immediately after collection	10.4
December			
Test 1	1.2	tested immediately after collection	6.0
Test 2	1.2	7.7 1 day	7.7

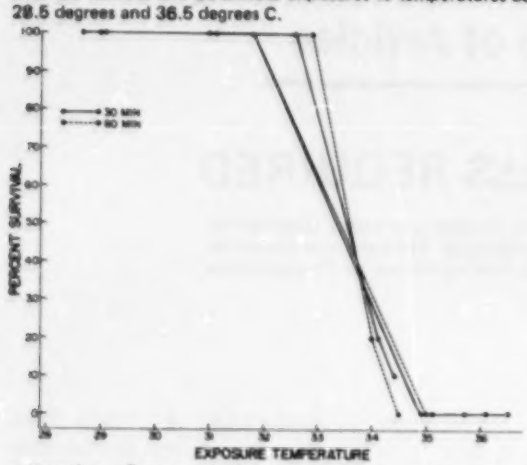
Table 2

The TL50 values (°C) for 30- and 60-minute high temperature exposures of *B. americanus* larvae collected in June, September, and December

Month	30-Min exposure	60-Min exposure
June		
Test 1	33.8	33.6[a]
Test 2	33.3[a]	33.4[a]
September	34.5	34.0
December		
Test 1	test temperatures too low	test temperatures too low
Test 2	33.3[a]	33.7

a Graphically extrapolated (data could not be processed by the Litchfield and Wilcoxon (1949) method).

Figure 3. Percentage survival of *Brachycentrus americanus* larvae after 30- minute and 60-minute exposures to temperatures between 28.5 degrees and 36.5 degrees C.



categories of upper lethal temperature response have been described (Precht 1967). The first category has no heat acclimation. The heat death temperature is not affected by temperature history. The second category, called reasonable heat acclimation, is the most common response for aquatic invertebrates (Precht *et al.* 1973). This is characterized by increases in heat death temperature with increased acclimation temperature. The third response is called paradoxical heat acclimation. Here the heat death temperature decreases with increasing acclimation temperature. This response is only rarely found (Precht *et al.* 1973, Al-Habbib and Grainger 1977).

In tests with *B. americanus* larvae the upper lethal temperature did not change between June, September, and December for both 30-minute and 60-minute exposures. Since the larvae were tested soon after collection, it can be assumed that heat tolerance is not affected by seasonal temperature change between 1 degree and 15 degrees C. These results place *B. americanus* in the no-heat-acclimation category.

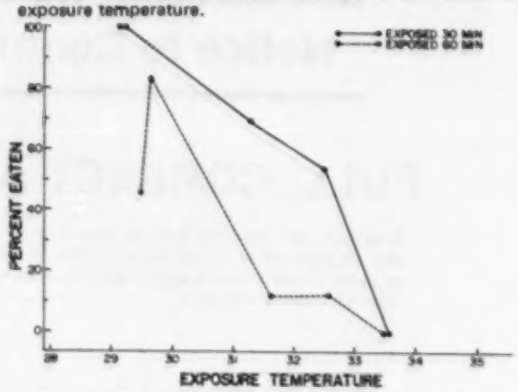
The feeding experiment indicated a behavioral effect from thermal shock. Two types of natural feeding behavior of *B. americanus* larvae were observed, as described by Gallepp (1977). Filtering, which is the spreading of the rear four legs radially about the case opening to catch food while attached facing into the current, was observed in the larvae that ate 100 percent of the offered brine shrimp. The term withdrawn is applied to another behavioral pattern in which the larvae are inside their cases, or resting in the case opening with the legs drawn up alongside the head. This behavior was observed in those larvae that did not eat any of the offered brine shrimp. Some of these larvae moved their

Table 3.

Mean survival TL50 (*B. americanus*) after 30- and 60-minute high temperature exposure and temperatures at which 50% of offered food was eaten

Item	30-Minute exposure	60-Minute exposure
Mean of the tolerance limits from June, September, and December 1976 tests (OC)	33.7	33.7
Temperatures at which 50% of the total brine shrimp are eaten 16 days after thermal shock of test 2 in December (OC)	32.1	30.1

Figure 4. Relationship between feeding of *B. americanus* larvae and exposure temperature.



bodies rapidly backward and forward in the case. The movement could be observed with the aid of a dissecting microscope. Similar behavior was reported by Gallepp (1977) in a few of his withdrawn larvae. Since the reduction in feeding occurred below the mean 30-minute and 60-minute tolerance limits (Table 3), the actual tolerance of these animals to temperature would be less if the larvae continued the withdrawn non-feeding behavior and eventually starved. Therefore, the effect of the shock would be delayed.

In summary, the 60-minute heat tolerance of *B. americanus* larvae does not change in response to seasonal water temperature changes of 1-15 degrees C. Simple survival counts of course, may be misleading. Feeding during a 16-day post-exposure period was affected at temperatures 3.6 degrees C lower than those that directly affected survival.

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